Listing of Claims

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims

1. (Original) A lentiviral vector comprising the following elements: a nucleic acid whose sequence includes (i) a functional packaging signal; (ii) a multiple cloning site (MCS); and (iii) at least one additional element selected from the group consisting of: a second MCS, a second MCS into which a heterologous nucleic acid is inserted, an HIV FLAP element, an expression-enhancing posttranscriptional regulatory element, a target site for a site-specific recombinase, and a self-inactivating (SIN) LTR, wherein the lentiviral vector is a lentiviral transfer plasmid or an infectious lentiviral particle.

2. (Original) The lentiviral vector of claim 1, wherein the vector comprises at least two elements selected from the group consisting of: a second MCS, a second MCS into which a heterologous nucleic acid is inserted, an HIV FLAP element, an expression-enhancing posttranscriptional regulatory element, a target site for a site-specific recombinase, and a self-inactivating (SIN) LTR.

3. (Original) The lentiviral vector of claim 1, wherein the vector comprises at least three elements selected from the group consisting of: a second MCS, a second MCS into which a heterologous nucleic acid is inserted, an HIV FLAP element, an expression-enhancing posttranscriptional regulatory element, a target site for a site-specific recombinase, and a self-inactivating (SIN) LTR.

4. (Original) The lentiviral vector of claim 1, wherein the vector comprises at least four elements selected from the group consisting of: a second MCS, a second MCS into which a heterologous nucleic acid is inserted, an HIV FLAP element, an expression-enhancing posttranscriptional regulatory element, a target site for a site-specific recombinase, and a self-inactivating (SIN) LTR.

5. (Original) The lentiviral vector of claim 1, wherein the vector comprises a second MCS, an HIV FLAP element, an expression-enhancing posttranscriptional regulatory element, a target site for a site-specific recombinase, and a self-inactivating (SIN) LTR.

6. (Original) The lentiviral vector of claim 1, wherein the vector comprises a second MCS into which a heterologous nucleic acid is inserted, an HIV FLAP element, an expression-enhancing posttranscriptional regulatory element, a target site for a site-specific recombinase, and a self-inactivating (SIN) LTR.

7. (Original) The lentiviral vector of claim 1, wherein the additional element is a second MCS.

8. (Original) The lentiviral vector of claim 1, wherein the additional element is a second MCS into which a heterologous nucleic acid is inserted.

9. (Original) The lentiviral vector of claim 1, wherein the vector has unique restriction sites for at least 4 enzymes selected from the group consisting of NotI, ApaI, XhoI, XbaI, HpaI, NheI, PacI, NsiI, SphI, Sma/Xma, AccI, BamHI, and SphI.

10. (Original) The lentiviral vector of claim 1, wherein the vector has unique restriction sites for at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, or at least 13 enzymes selected from the group consisting of NotI, ApaI, XhoI, XbaI, HpaI, NheI, PacI, NsiI, SphI, Sma/Xma, AccI, BamHI, and SphI.

11. (Original) The lentiviral vector of claim 1, wherein the additional element is an HIV FLAP element.

12. (Original) The lentiviral vector of claim 1, wherein the additional element is an expression-enhancing posttranscriptional regulatory element.

13. (Original) The lentiviral vector of claim 12, wherein the expression-enhancing posttranscriptional regulatory element is a WRE.

14. (Original) The lentiviral vector of claim 1, wherein the additional element is a target site for a site-specific recombinase.

15. (Original) The lentiviral vector of claim 14, wherein the site is a loxP site.

16. (Original) The lentiviral vector of claim 1, wherein the lentiviral vector is a lentiviral transfer plasmid.

17. (Original) The lentiviral transfer plasmid of claim 16, wherein the plasmid has a size of less than 10 kB.

18. (Original) The lentiviral transfer plasmid of claim 16, wherein the plamid has a size of less than 9 kB.

19. (Original) The lentiviral transfer plasmid of claim 16, wherein the plasmid has a size of less than 8 kB.

20. (Original) The lentiviral transfer plasmid of claim 16, wherein the plasmid has a size of less than 7 kB.

21. (Original) The lentiviral transfer plasmid of claim 16, wherein the plasmid has a size of approximately 6 kB.

22. (Original) The lentiviral vector of claim 1, wherein the lentiviral vector is an infectious lentiviral particle.

23. (Original) The lentiviral vector of claim 1, further comprising: a heterologous promoter or promoter-enhancer.

24. (Original) The lentiviral vector of claim 23, wherein the heterologous promoter or promoter-enhancer is selected from the group consisting of: the CMV promoter, the CMV promoter-enhancer, and the ubiquitin C promoter.

25. (Original) The lentiviral vector of claim 24, wherein the heterologous promoter is an inducible promoter.

26. (Original) The lentiviral vector of claim 24, wherein the heterologous promoter is a cell type specific or tissue specific promoter.

27. (Original) The lentiviral vector of claim 23, wherein the heterologous promoter is an RNA polymerase promoter.

28. (Original) The lentiviral vector of claim 27, wherein the RNA polymerase promoter is an RNA polymerase III promoter.

29 (Original) The lentiviral vector of claim 28, wherein the RNA polymerase III promoter is a U6 promoter.

30. (Original) The lentiviral vector of claim 28, wherein the RNA polymerase III promoter is an H1 promoter.

31. (Original) The lentiviral vector of claim 27, wherein the RNA polymerase promoter is an RNA polymerase II promoter.

32. (Original) The lentiviral vector of claim 23, further comprising a second heterologous promoter or promoter-enhancer.

33. (Original) The lentiviral vector of claim 1, further comprising a heterologous nucleic acid encoding a selectable marker operably linked to a promoter.

34. (Original) The lentiviral vector of claim 1, further comprising a heterologous nucleic acid encoding a reporter molecule operably linked to a promoter.

35. (Original) The lentiviral vector of claim 34, wherein the reporter molecule is selected from the group consisting of: GFP, EGFP, dsRed, dsRed2, cyan fluorescent protein, yellow fluorescent protein, blue fluorescent protein, dsRed, dsRed2, luciferase, and aequorin.

36. (Original) The lentiviral vector of claim 34, further comprising an RNA polymerase promoter.

37. (Original) The lentiviral vector of claim 36, wherein the RNA polymerase promoter is an RNA polymerase III promoter.

38. (Original) The lentiviral vector of claim 1, wherein the lentiviral vector is a transfer plasmid, further comprising a genetic element sufficient for stable maintenance of the transfer plasmid as an episome within mammalian cells.

39-57. (Canceled)

58. (Original) A cell comprising the lentiviral vector of claim 1.

59. (Original) The cell of claim 58, wherein the cell comprises a nucleic acid or nucleic acids having sequences encoding Gag, Pol, and Env proteins.

60. (Original) A cell comprising a provirus derived from the lentiviral vector of claim 1.

61-123.(Canceled)

124. (Previously presented) A kit comprising (a) a lentiviral transfer plasmid comprising a nucleic acid sequence including (i) a functional packaging signal; (ii) a multiple cloning site (MCS) into which a heterologous gene may be inserted; and (iii) at least one additional element selected from the group consisting of: a second MCS, an HIV FLAP element, a heterologous promoter, a heterologous enhancer, an expression-enhancing posttranscriptional regulatory element, a target site for a site-specific recombinase, and a self-inactivating (SIN) LTR; and one or more of the following items: (b) a packaging mix comprising one or more plasmids that collectively provide nucleic acid sequences coding for retroviral or lentiviral Gag and Pol proteins and an envelope protein; (c) cells (e.g., a cell line) that are permissive for production of lentiviral particles such as 293T cells; (d) packaging cells, e.g., a cell line that is permissive for production of lentiviral particles and provides the proteins Gag, Pol, Env, and, optionally, Rev; (e) cells suitable for use in titering lentiviral particles; a transfection-enhancing agent such as Lipofectamine; (f) a selection agent such as an antibiotic, preferably corresponding to an antibiotic resistance gene in the lentiviral transfer plasmid; (g) instructions for use; (h) a positive control plasmid.

125. (Previously presented) The lentiviral vector of claim 1, wherein the functional packaging signal is Psi.

126. (Previously presented) The lentiviral vector of claim 1, wherein the site-specific recombinase is Cre.

127. (Previously presented) The lentiviral vector of claim 1, wherein the target site for the site-specific recombinase is LoxP.

128. (Previously presented) The lentiviral vector of claim 1, wherein the first or second MCS comprises target sites for ApaI, XbaI, HpaI, NpeI, PacI, and XhoI.

129. (Previously presented) The lentiviral vector of claim 1, wherein the first or second MCS comprises target sites for NotI, NsiI, SphI, SmaI/XmaI, AscI, BamHI, and EcoRI.

130. (Previously presented) The lentiviral vector of claim 34, wherein the reporter is selected from the group consisting of EGFP and dsRed2.

131. (Canceled)

132. (Previously presented) The lentiviral vector of claim 1, wherein the promoter is selected from the group consisting of a cytomegalovirus (CMV) promoter, a Ubiquitin C (UbC) promoter, a polymerase III promoter, and a U6 promoter.

133-135. (Canceled)

136. (Previously presented) The lentiviral vector of claim 1, wherein the lentiviral vector has a size of approximately 5,830 base pairs.

137. (Previously presented) The lentiviral vector of claim 1, wherein the lentiviral vector has a size of approximately 6,700 base pairs.

138. (Previously presented) The lentiviral vector of claim 1, wherein the lentiviral vector has a size of approximately 6,750 base pairs.

139. (Previously presented) The lentiviral vector of claim 1, wherein the lentiviral vector has a size of approximately 7,250 base pairs.

140. (Previously presented) The lentiviral vector of claim 1, wherein the lentiviral vector has a size of approximately 7,350 base pairs.

141. (Previously presented) The lentiviral vector of claim 1, wherein the lentiviral vector has a size of approximately 7,650 base pairs.

142. (Previously presented) The lentiviral vector of claim 1, wherein the lentiviral vector has a size of approximately 7,925 base pairs.

143. (Previously presented) The lentiviral vector of claim 1, wherein the lentiviral vector has a size of approximately 7,975 base pairs.

144. (Previously presented) The lentiviral vector of claim 1, wherein the expression-enhancing posttranscriptional regulatory element is a woodchuck hepatitis regulatory element (WRE).

145. (Previously presented) The cell of claim 58, wherein the cell is selected from the group consisting of a $CD8^+$ E10 thymoma cell, a primary mouse splenocyte, a 293 T cell, an AK7 ES cell, a D7 cell, a cell harvested from spleen, and a cell harvested from lymph node.

146-151. (Canceled)

152. (Previously presented) A lentiviral vector comprising the following elements: a nucleic acid whose sequence includes (i) a Psi packaging signal; (ii) two multiple cloning sites; (iii) an HIV FLAP element; (iv) a Woodchuck Hepatitis Regulatory Element (WRE); (v) a target site for Cre recombinase; and (vi) a self-inactivating (SIN) LTR;

wherein the lentiviral vector is a lentiviral transfer plasmid or an infectious lentiviral particle.

153. (Previously presented) A lentiviral vector comprising the following elements: a nucleic acid whose sequence includes (i) a Psi packaging signal; (ii) two multiple cloning sites; (iii) an HIV FLAP element; (iv) a Woodchuck Hepatitis Regulatory Element (WRE); (v) a target site for Cre recombinase; (vi) a self-inactivating (SIN) LTR; and (vii) a promoter selected from the group consisting of a cytomegalovirus (CMV) promoter, a polymerase

III promoter, a U6 promoter, and a Ubiquitin C (UbC) promoter;

wherein the lentiviral vector is a lentiviral transfer plasmid or an infectious lentiviral particle.

154-156. (Canceled)